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(54) Title: A METHOD OF TREATING CANCER

(57) Abstract

The present invention relates to a method of treating cancer which comprises administering to a mammalian patient a compound which inhibits Raf and a compound which inhibits farnesyl protein transferase.

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TITLE OF THE INVENTION
A METHOD OF TREATING CANCER

BACKGROUND OF THE INVENTION

The present invention relates to a method of treating cancer using a combination of a compound which has Raf antagonist activity and a compound which has farnesyl transferase inhibiting activity.

The Raf antagonist compounds used in the present invention demonstrate anti-cancer activity through antagonism of the kinase, Raf . The *raf* genes code for a family of proteins which can be oncogenically activated through N-terminal fusion, truncation or point mutations. Raf is a member of the MAP Kinase cascade, which also includes MEK's and MAP Kinase (ERK). Raf can be activated and undergoes rapid phosphorylation in response to treatment of cells with PDGF, EGF, insulin, thrombin, endothelin, acidic FGF, CSF1 or TPA, as well as in response to oncoproteins v-fms, v-src, v-sis, Hras and polyoma middle T antigen. Antisense constructs which reduce cellular levels of c-Raf, and hence Raf activity, inhibit the growth of oncogene-transformed rodent fibroblasts in soft agar, while exhibiting little or no general cytotoxicity. Since inhibition of growth in soft agar is highly predictive of tumor responsiveness in whole animals, these studies suggest that the antagonism of Raf is an effective means by which to treat cancers in which Raf plays a role.

Examples of cancers where Raf is implicated through overexpression include cancers of the brain, genitourinary tract, lymphatic system, stomach, larynx and lung. More particularly, such examples include histiocytic lymphoma, lung adenocarcinoma and small cell lung cancers. Additional examples include cancers in which overexpression or activation of Raf-activating oncogenes (e.g., K-*ras*, *erb*-B) is observed. More particularly, such cancers include pancreatic and breast carcinoma.

The Ras protein is part of a signalling pathway that links cell surface growth factor receptors to nuclear signals initiating cellular proliferation. Biological and biochemical studies of Ras action

indicate that Ras functions like a G-regulatory protein. In the inactive state, Ras is bound to GDP. Upon growth factor receptor activation, Ras is induced to exchange GDP for GTP and undergoes a conformational change. The GTP-bound form of Ras propagates the growth stimulatory signal until the signal is terminated by the intrinsic GTPase activity of Ras, which returns the protein to its inactive GDP bound form (D.R. Lowy and D.M. Willumsen, *Ann. Rev. Biochem. 62*:851-891 (1993)). Activation of Ras leads to activation of multiple intracellular signal transduction pathways, including the MAP Kinase pathway and the Rho/Rac pathway (Joneson *et al.*, Science *271*:810-812).

Mutated *ras* genes are found in many human cancers, including colorectal carcinoma, exocrine pancreatic carcinoma, and myeloid leukemias. The protein products of these genes are defective in their GTPase activity and constitutively transmit a growth stimulatory signal.

The Ras protein is one of several proteins that are known to undergo post-translational modification. Farnesyl-protein transferase utilizes farnesyl pyrophosphate to covalently modify the Cys thiol group of the Ras CAAX box with a farnesyl group (Reiss *et al.*, *Cell, 62*:81-88 (1990); Schaber *et al.*, *J. Biol. Chem., 265*:14701-14704 (1990); Schafer *et al.*, *Science, 249*:1133-1139 (1990); Manne *et al.*, *Proc. Natl. Acad. Sci USA, 87*:7541-7545 (1990)).

Ras must be localized to the plasma membrane for both normal and oncogenic functions. At least 3 post-translational modifications are involved with Ras membrane localization, and all 3 modifications occur at the C-terminus of Ras. The Ras C-terminus contains a sequence motif termed a "CAAX" or "Cys-Aaa^1-Aaa^2-Xaa" box (Cys is cysteine, Aaa is an aliphatic amino acid, the Xaa is any amino acid) (Willumsen *et al.*, *Nature 310*:583-586 (1984)). Depending on the specific sequence, this motif serves as a signal sequence for the enzymes farnesyl-protein transferase or geranylgeranyl-protein transferase, which catalyze the alkylation of the cysteine residue of the

CAAX motif with a C_{15} or C_{20} isoprenoid, respectively. (S. Clarke., *Ann. Rev. Biochem. 61*:355-386 (1992); W.R. Schafer and J. Rine, *Ann. Rev. Genetics 30*:209-237 (1992)). However, direct inhibition of farnesyl-protein transferase would be more specific and attended by fewer side effects than would occur with the required dose of a general inhibitor of isoprene biosynthesis.

Other farnesylated proteins include the Ras-related GTP-binding proteins such as Rho, fungal mating factors, the nuclear lamins, and the gamma subunit of transducin. James, et al., *J. Biol. Chem. 269*, 14182 (1994) have identified a peroxisome associated protein Pxf which is also farnesylated. James, et al., have also suggested that there are farnesylated proteins of unknown structure and function in addition to those listed above.

Inhibitors of farnesyl-protein transferase (FPTase) have been described in two general classes. The first class includes analogs of farnesyl diphosphate (FPP), while the second is related to protein substrates (e.g., Ras) for the enzyme. The peptide derived inhibitors that have been described are generally cysteine containing molecules that are related to the CAAX motif that is the signal for protein prenylation. (Schaber *et al., ibid*; Reiss *et. al., ibid*; Reiss *et al., PNAS, 88*:732-736 (1991)). Such inhibitors may inhibit protein prenylation while serving as alternate substrates for the farnesyl-protein transferase enzyme, or may be purely competitive inhibitors (U.S. Patent 5,141,851, University of Texas; N.E. Kohl *et al., Science, 260*:1934-1937 (1993); Graham, et al., *J. Med. Chem., 37*, 725 (1994)).

Inhibition of farnesyl-protein transferase has been shown to block the growth of *ras*-transformed cells in soft agar and to modify other aspects of their transformed phenotype. It has also been demonstrated that certain inhibitors of farnesyl-protein transferase selectively block the processing of the Ras oncoprotein intracellularly (N.E. Kohl *et al., Science, 260*:1934-1937 (1993) and G.L. James *et al., Science, 260*:1937-1942 (1993). Recently, it has been shown that an inhibitor of farnesyl-protein transferase blocks the growth of *ras*-dependent tumors in nude mice (N.E. Kohl *et al., Proc. Natl. Acad. Sci*

U.S.A., *91*:9141-9145 (1994) and induces regression of mammary and salivary carcinomas in *ras* transgenic mice (N.E. Kohl *et al.*, *Nature Medicine*, 1:792-797 (1995).

Indirect inhibition of farnesyl-protein transferase *in vivo* has been demonstrated with lovastatin (Merck & Co., Rahway, NJ) and compactin (Hancock *et al.*, *ibid*; Casey *et al.*, *ibid*; Schafer *et al.*, *Science 245*:379 (1989)). These drugs inhibit HMG-CoA reductase, the rate limiting enzyme for the production of polyisoprenoids including farnesyl pyrophosphate. Inhibition of farnesyl pyrophosphate biosynthesis by inhibiting HMG-CoA reductase blocks Ras membrane localization in cultured cells.

A Raf antagonist compound and a farnesyl protein transferase inhibitor are used in the present invention to treat cancer, such as in tumor cells which are not particularly Raf or FPTase dependent. The Raf antagonist compound and a farnesyl protein transferase inhibiting compound are used in combination.

SUMMARY OF THE INVENTION

A method of treating cancer is disclosed which is comprised of administering to a mammalian patient in need of such treatment an effective amount of a Raf antagonist compound and an effective amount of a farnesyl protein transferase inhibiting compound.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method of treating cancer which is comprised of admininstering to a mammalian patient in need of such treatment an effective amount of a Raf antagonist compound and an effective amount of a farnesyl protein transferase inhibiting compound. Any compound which antagonizes Raf and any compound which inhibits farnesyl protein transferase can be used.

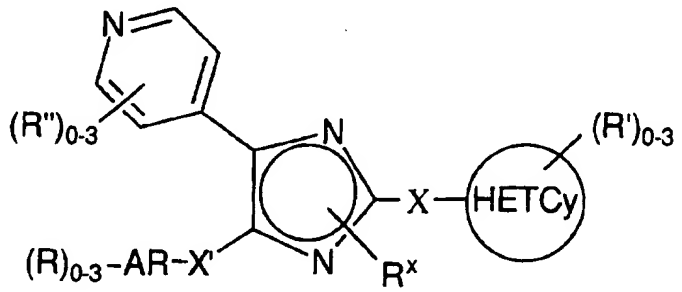
As used herein the term Raf antagonist is used in the general sense to relate to compounds which antagonize, inhibit or counteract the activity of the *raf* gene or the protein produced in response thereto.

The term farnesyl protein transferase inhibiting compound is likewise used in the general sense and refers to compounds which antagonize, inhibit or counteract the activity of the gene coding farnesyl protein transferase or the protein produced in response thereto.

Cancers which are treatable in accordance with the invention described herein include cancers of the brain, genitourinary tract, lymphatic system, stomach, larynx, liver and lung. More particularly, such cancers include histiocytic lymphoma, lung adenocarcinoma and small cell lung cancers. Additional examples include cancers in which overexpression or activation of Raf-activating oncogenes (e.g., K-*ras*, *erb*-B) is observed. More particularly, such cancers include pancreatic, mammary and salivary carcinomas, colorectal carcinoma, exocrine pancreatic carcinoma and myeloid leukemias.

Examples of compounds which antagonize Raf are as follows:

(a) a compound represented by formula (I-a):



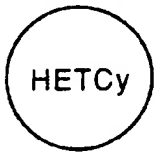
(I-a)

or a pharmaceutically acceptable salt thereof, wherein:

AR represents an aromatic group containing 6-10 atoms;

X and X' each independently represent $-(CH_2)_m-Y-(CH_2)_n-$, wherein m and n represent integers within the range of from 0 - 4, such that the sum of m and n is from 0 - 6; Y represents a member selected from the group consisting of: a direct bond: O; $S(O)_y$, with y equal to

0, 1 or 2; $NR^{q'}$, with $R^{q'}$ as defined below; C(O); OC(O); C(O)O; $SO_xNR^{q'}$ with x equal to 1 or 2 and $R^{q'}$ as defined below; $NR^{q'}SO_x$; $C(O)NR^{q'}$ and $NR^{q'}C(O)$;



represents a 4 to 10 membered non-aromatic heterocycle containing at least one N atom, and optionally containing 1-2 additional N atoms and 0-1 O or S atom;

R^x represents H, C_{1-6} alkyl$(R^q)_3$, OC_{1-6} alkyl$(R^q)_3$ or $C(O)C_{1-6}$ alkyl$(R^q)_3$;

each R and R" independently represents a member selected from the group consisting of: halo; hydroxy; C_{1-6} alkyl$(R^q)_3$; OC_{1-6} alkyl$(R^q)_3$; C_{3-8} cycloalkyl$(R^q)_3$; CN; $CONH_2$; $CONHC_{1-6}$ alkyl$(R^q)_3$; $CON(C_{1-6}$ alkyl$(R^q)_3)_2$; NH_2; NHC_{1-6} alkyl$(R^q)_3$; $N(C_{1-6}$ alkyl$(R^q)_3)_2$; CO_2H; CO_2C_{1-6} alkyl$(R^q)_3$; $C(O)C_{1-6}$ alkyl$(R^q)_3$; aryl$(R^q)_3$; heteroaryl$(R^q)_3$; CF_3; SH; NO_2; SO_yC_{1-6} alkyl$(R^q)_3$, with y as defined above; SO_2NH_2; SO_2NHC_{1-6} alkyl$(R^q)_3$; $SO_2N(C_{1-6}$ alkyl$(R^q)_3)_2$; $NHSO_2C_{1-6}$alkyl$(R^q)_3$, $NHSO_2$aryl$(R^q)_3$, $NHSO_2$heteroary$(R^q)_3$, $N(R^{q'})C(O)C_{1-6}$ alkyl$(R^q)_3$; $NR^{q'}C(O)NH$ $(C_{1-6}$ alkyl$(R^q)_3)$;
C_{2-4} alkenyl$(R^q)_{2-3}$ and C_{2-4} alkynyl$(R^q)_{1-3}$;

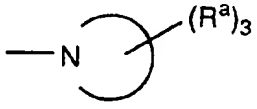
each R' independently represents a member selected from the group consisting of: $CONH_2$; $CONHC_{1-6}$ alkyl$(R^q)_3$; $CON(C_{1-6}$ alkyl$(R^q)_3)_2$; $CONHC_{3-8}$ cycloalkyl$(R^q)_3$; $CON(C_{3-8}$ cycloalkyl$(R^q)_3)_2$; CO_2H; CO_2C_{1-6} alkyl$(R^q)_3$; $C(O)C_{1-6}$ alkyl$(R^q)_3$; CO_2C_{3-8} cycloalkyl$(R^q)_3$; $C(O)C_{3-8}$ cycloalkyl$(R^q)_3$; $-[C(O)(CH_2)_j\text{-}CR^5R^6\text{-}(CH_2)_k\text{-}NR^7]_p\text{-}R^8$; $-C(O)C_{3-8}$ cycloalkyl$(R^q)_3$; -C(O)heterocyclyl$(R^q)_3$; $CON[C_{1-6}$alkyl$(R^q)_3][C_{3-8}$ cycloalkyl$(R^q)_3]$; C(O)aryl$(R^q)_3$, C(O)heteroaryl$(R^q)_3$;

wherein p represents 1, 2 or 3;
j and k are integers independently selected from 0 - 3;

each R^5 and R^6 independently represents H, aryl, C_{1-6} alkyl$(R^q)_3$, or each CR^5R^6 taken in combination represents a 3, 4, 5 or 6 membered cycloalkyl or heterocyclyl group, an aryl group or a heteroaryl group, wherein when p equals 1, at least one of j and k is 1, 2 or 3;

each R^7 and R^8 independently represents H, C_{1-6} alkyl or aryl;

R^q represents a member selected from the group consisting of: $R^{q'}$; CN; CO_2H; CO_2C_{1-4} alkyl; $C(O)C_{1-4}$ alkyl ; aryl$(R^a)_3$; NH_2; NHC_{1-6} alkyl$(R^a)_3$; $N(C_{1-6}$ alkyl$(R^a)_3)_2$; heteroaryl$(R^a)_3$; $CONH_2$; SH ; $S(O)_y$ C_{1-6} alkyl$(R^a)_3$; $C(O)NHC_{1-6}$ alkyl$(R^a)_3$; $C(O)N(C_{1-6}$ alkyl$(R^a)_3)_2$; -heteroalkyl$(R^a)_3$; -$NHC(O)NH_2$; -$NHC(NH)NH_2$;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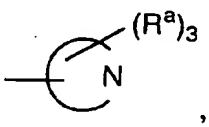
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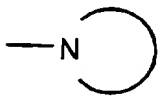
wherein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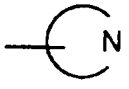
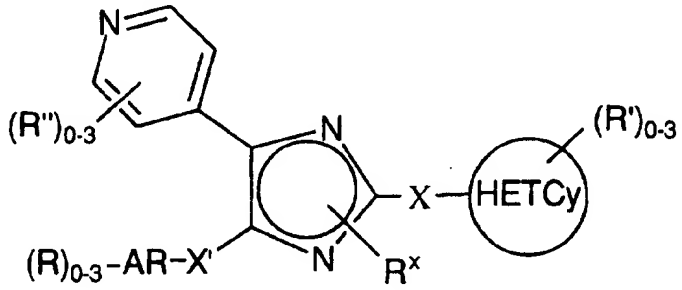

independently represent mono or bicyclic ring systems, non-aromatic or partially aromatic, containing from 5-10 ring atoms, 1-4 of which are N and 0-1 of which are O or $S(O)_y$, with y equal to 0, 1 or 2, optionally containing 1-2 carbonyl groups;

each R^a independently represents a member selected from the group consisting of: H, C_{1-6} alkyl, OC_{1-6} alkyl, aralkyl, substituted aralkyl, heteroaralkyl, substituted heteroaralkyl, aralkoxy, substituted

aralkoxy, halo, hydroxy, CN, $CONH_2$, $CONHC_{1-6}$ alkyl, $CON(C_{1-6}$ alkyl$)_2$, CO_2H, CO_2C_{1-6} alkyl, $C(O)C_{1-6}$ alkyl, phenyl, CF_3, SH, NO_2, SO_yC_{1-6} alkyl, with y as defined above; SO_2NH_2, SO_2NHC_{1-6} alkyl, $NHSO_2$(substituted aryl), $NHSO_2$(substituted heteroaryl), $NHSO_2C_{1-6}$alkyl, $NHSO_2$aryl, $NHSO_2$heteroaryl, NH_2, NHC_{1-6} alkyl, $N(C_{1-6}$ alkyl$)_2$, $NHC(O)C_{1-6}$ alkyl, $NHC(O)NH(C_{1-6}$ alkyl), C_{2-4} alkenyl and C_{2-4} alkynyl;

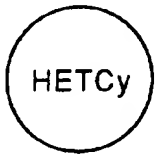
and $R^{q'}$ represents H, OH, C_{1-4} alkyl, $-OC_{1-4}$ alkyl, aryl or $C(O)C_{1-4}$ alkyl;

(b) a compound represented by formula (I-b)

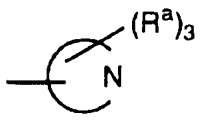


(I-b)

or a pharmaceutically acceptable salt thereof, wherein:

AR, X, X', x, Y, y, $R^{q'}$, 

, R^x, R, R'', j, k, R^7, R^8,

and


are as defined above with respect to formula (I-a);